学校编码：**10384** 分类号______密级_____

学　　号：**20520070153604** **UDC**_____

厦门大学

博　士　学　位　论　文

DNA 分子构象和电子传递性能的应用研究

Applications of Conformation and Electron Transfer Properties of DNA

林玲玲

指导教师姓名：林仲华　　教　授

　　　　　　　周剑章　　副教授

专 业 名 称：物　理　化　学

论文提交日期：2010 年 12 月

论文答辩日期：2010 年 12 月

学位授予日期：2011 年　　月

答辩委员会主席：__________

评　　阅　　人：__________

2010 年 12 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行)》。

另外,该学位论文为(　　　　　　　　　　　　　　　　)课题(组)的研究成果，获得（　　　　　　　　　）课题（组）经费或实验室的资助，在（　　　　　　　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）:

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打"√"或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

摘 要

DNA 分子构象和电子传递性能的应用研究

生物大分子，特别是DNA分子，由于其特殊的结构和特异性识别功能被广泛用于分子识别、结构组装和器件设计开发等方面的研究，并展示出独特的优势。DNA构象的形变性质，特别是DNA双螺旋的刚性弯曲和单链DNA片段、错配和一些特殊DNA构象异常的柔韧性，备受物理学家、化学家和生物学家的关注，并被广泛应用于分子识别过程的研究和模拟。在DNA纳米杂合体系中，DNA形态的多变性、可设计的特殊序列的识别性和各种各样的物理化学性质在基于核酸的纳米结构、纳米传感器和纳米器件的开发应用中显示出极大的优势。备受科学家关注的DNA独特的电子传递性质也成为分子生物学和纳米科学中最为活跃的研究领域之一，它为纳米器件的制作提供了一种新技术、新方法，对分子级电子元件的研究具有深远的意义。电化学及光学等研究方法为DNA性质的研究及应用提供了有力的手段。

本文利用电化学和光学方法研究DNA构象变化及电子传递性能，同时结合纳米技术构建纳米/生物一体化体系，开发基于DNA及纳米/生物一体化体系的传感器及信号可控的光电体系，主要进行了以下几个方面的工作：（1）基于DNA双螺旋的形变性质和选择合适的电位范围及电化学标记物，利用超微电极和低电流检测法设计了高灵敏的DNA特异性检测传感器；（2）研究了不同pH条件下Fe^{3+}诱导的ds/ssDNA大分子三级构象的变化及其对AuNPs SPR吸收的影响，并对其机理做了相应研究；（3）以PCA、PCA-ssDNA及PCA-dsDNA为组装分子，研究PCA/TiO_2、ssDNA-PCA/TiO_2及dsDNA-PCA/TiO_2纳米体系的光电响应，讨论各种组装分子对TiO_2光生电子空穴在界面分离及电子传递过程的影响。主要取得以下结果：

一．高灵敏的 DNA 杂交及假阳性杂交的电化学检测

1. 利用DNA在电极界面的取向以及完全匹配DNA双链的刚性和含错配碱基DNA双链特殊的柔韧性，通过分析Fc-DNA分子层线性扫描伏安响应阳极峰的峰电流、峰电位与扫描速度的关系，区分DNA的完全匹配杂交与假阳性杂交，发现假阳性杂交形成的Fc-DNA的伏安响应阳极峰电位（E_{pa}）比起完全匹配的Fc-DNA明显负移，且峰电流与扫描速度的比值比起完全匹配的Fc-DNA分子层更大，利用这一性质提高了DNA分子检测的选择性。

2. 对所设计的DNA传感器靶标DNA分子的检测限测试表明，本方法可达到 100 fM的检测限，在 1 nM到 100 fM的靶标DNA浓度内，Fc-DNA分子层线性伏安响应的阳极峰电流与靶标DNA浓度的对数成线性关系。该方法可以较容易地检测到 6.09×10^{6} 个dsDNA分子，这使得该方法有可能直接应用于遗传病、基因变异或传染病的识别。

二．不同pH下Fe^{3+}诱导的DNA构象变化及其对Au纳米粒子聚集行为的控制

1. 利用UV吸收光谱、CD光谱、Zeta电位和拉曼光谱方法研究不同pH条件下Fe^{3+}诱导的ds/ss小牛胸腺DNA三级构象的变化，以及Fe^{3+}与ds/ssDNA在不同pH条件下相互作用的位点及相互作用的方式。实验结果表明，在pH 6.0 条件下，Fe^{3+}主要作用在DNA的磷酸骨架上；而在pH 8.0 条件下，DNA碱基环上的N原子成为与Fe^{3+}作用更有效的配体。Fe^{3+}与ds/ssDNA相互作用方式的改变将会使ds/ssDNA的三级构象发生变化，即当pH从碱性变化到酸性时ds/ssDNA将实现从伸长的螺旋态到折叠的堆积态之间的转变。

2. 测试了不同Fe^{3+}浓度及pH下Fe^{3+}-ds/ss小牛胸腺DNA构象变化对AuNPs聚集行为的影响。实验结果表明AuNPs可以有效地区分伸长的螺旋态和折叠的堆积态DNA，因此Fe^{3+}-ds/ssDNA/AuNPs杂合体系有望作为DNA三级构象变化的显色探针；Fe^{3+}-dsDNA/AuNPs杂合体系的A_{650}/A_{520}值在pH 10.0~7.0 范围内与pH值的变化成线性关系；而Fe^{3+}-ssDNA/AuNPs杂合体系的A_{650}/A_{520}则在一个很窄的pH范围内对体系的pH变化极为灵敏，即当pH从 8.0 变化到 6.5 时，体系的A_{650}/A_{520}值迅速增加。该实验结果意味着Fe^{3+}-ssDNA/AuNPs杂合体系将有望应

用于生物体系的pH检测。

三．PCA、DNA分子组装的TiO_2光电体系的研究

发现通过组装分子，结合外加电位和激发光波长控制，可以调控TiO_2纳米管阵列的光电化学行为，尤其是光电流方向。

1. 不组装分子时，TiO_2纳米管阵列只能产生阳极光电流

2. 组装PCA分子和ssDNA-PCA分子后，TiO_2纳米管阵列可以通过调控外加电位产生阳极或阴极光电流。

3. 组装dsDNA-PCA分子后，TiO_2纳米管阵列可以通过调控外加电位产生阳极或阴极光电流；可以同时通过调控外加电位和激发光波长，在带光激发下产生阳极光电流，在亚带光激发下产生阴极光电流。

关键词：DNA；传感器；金纳米粒子；TiO_2纳米管；电子传递；光电化学

Abstract

Applications of Conformation and Electron Transfer Properties of DNA

Biomolecules，particularly DNA molecules, thanks to their special structure and intrinsic recognition specificity, have been widely used for molecular recognition, structure assembly and device design with unique advantages. The deformability of DNA, in particular, the bending rigidity of double helix of DNA, the anomalous flexibility of its single-stranded segments, mismatches and other peculiar DNA conformations, have been well-understood and widely used for studying and modeling molecular recognition processes by physicists, chemists and biologists. The deformational polymorphism, the programmable sequence-specific recognition and various physicochemical natures of nucleic acids have shown great advantages in nucleic acids-based nanostructures, nanosensors and nanodevices fabrications in terms of their applicabilities. Meanwhile, the unique electron transfer property of DNA is one of the most active fields of research in molecular biology and nano science. It provides a new approach to nano device and is of profound significance for developing molecular electronic device. The methodology from the point view of electrochemistry and optics provides a powerful alternative to investigate and apply the unique properties of DNA.

In this paper, we have studied the DNA conformational changes and electron transfer performance via electrochemical and optical methods. The unique properties of DNA were combined with nanotechnology for constructing nano/biological hybridized system. Based on this nano/biological hybridized system, DNA biosensors and signal-controllable photoelectric systems were designed. The work mainly included the following aspects: (1) Based the deformability of DNA double helix, we constructed a highly sensitive electrochemical sensor for detection of sequence-specific DNA with appropriate potential scope and electrochemical markers via ultramicroelectrode, low current voltammetry. (2) We investigated the conformational changes of giant ds/ssDNA driven by Fe^{3+} at different pH and

revealed for the first time its effects on the SPR of AuNPs. Zeta potentials and Raman spectra were studied for understanding the interaction mechanism between Fe^{3+} and ds/ssDNA as well as that between AuNPs and DNA (ds/ssDNA and Fe^{3+}-ds/ssDNA). (3) The photoelectric response of PCA/TiO_2, ssDNA-PCA/TiO_2 and dsDNA-PCA/TiO_2 nano systems were investigated using the assembled molecules, namely PCA, PCA-ssDNA and PCA-dsDNA, and the specific influences of those assembled molecules on the photoproduced electron-hole separation of TiO_2 in the interface and the electron transfer process effects were also disscussed.

The main achievements are shown below:

1. Highly sensitive electrochemical detection of sequence-specific DNA

(1) Based on the molecular orientation of DNA helices on the electrode surface and the deformability difference between the DNA with the fully complementary double helix and those with mismatched helix, the fully complementary hybridization could be distinguished from false positive hybridization through the peak potentials and peak currents dependence on scan rate using the linear potential sweep voltammetry. The anodic peak potentials (E_{pa}) of voltammograms recorded for false positive hybridization shifted more negatively than its counterpart and as well, a larger ratio of peak currents to scan rate was also observed for the false positive hybridization. Discoveries of such properties were employed to improve the selectivity of DNA.

(2) The minimal concentration of target DNA detectable by the as-designed device was found to be 100 fM. The anodic peak currents were linearly dependent on the target DNA concentrations in log scales in the range of 1 nM to 100 fM. Measurement of dsDNA molecules as sensitive as 6.09×10^6 could be facily realized through this method based on ultramicroelectrode and low current voltammetry. That is, the methord might be sensitive enough to identify hereditary diseases, genetic abnormalities or infections directly.

2. pH-dependent conformational change of Fe^{3+}-binding giant DNA for control of aggregation of gold nanoparticles

(1) We investigated the pH-dependent conformational changes of ds/ssDNA driven by Fe^{3+} using CD spectroscopy. Raman spectra and ζ-potentials provided evidences of interactions between Fe^{3+} with ds/ssDNA as well as that between AuNPs with DNA (ds/ssDNA, Fe^{3+}-ds/ssDNA) which are pH-regulated. Fe^{3+} can bind at purine nitrogen atoms of ds/ssDNA at basic pH; whereas at acidic pH, Fe^{3+} prefers to bind at phosphate groups. Binding of phosphate groups with Fe^{3+} at acidic pH would lead to the conformational changes of Fe^{3+}-ds/ssDNA from elongated coil state to folded compact state.

(2) To investigate the aggregation of Fe^{3+}-ctDNA/AuNPs, a series of detections on color changes of Fe^{3+}-ctDNA/AuNPs as a function of Fe^{3+} concentration and the pH were undertaken. The colorimetric behaviors of AuNPs were demonstrated sensitive to the conformational changes of Fe^{3+}-ds/ssDNA at different pH, which could be applied for assaying the conformational changes of DNA. The assay based on the Fe^{3+}-dsDNA provides an advantage in terms of detection in a wide pH range from 6.0 to 10.6. On the contrary, Fe^{3+}-ssDNA is very sensitive in a narrow range i.e., from pH 6.0 to 7.5, which might be applied for detectionsin a biological relevant pH range.

3. Research of TiO_2 photoelectric system assembled with PCA, DNA molecule

The photoelectric chemical behavior of TiO_2 nanotubes array, especially the photocurrent direction, could be controlled by assembling molecules together with controlling the applied potential and the wavelength of the light

(1) TiO_2 nanotubes array with no molecule assembled generated only anode photocurrent.

(2) TiO_2 nanotubes array assembled with PCA molecules and ssDNA-PCA molecule could generate anode or cathodic photocurrent bycontrolling the applied potential.

(3) TiO_2 nanotubes array assembled with dsDNA-PCA molecules could generate anode or cathodic photocurrent by controlling the applied potential. Moreover, through simultaneous control of the applied potential and the wavelength of light, TiO_2 nanotubes array assembled with dsDNA-PCA molecules could generate anode photocurrent by band-gap light excitation and generate cathodic photocurrent by

sub-band-gap light excitation.

Keywords： DNA, sensor, AuNPs, TiO_2 nanotubes, electron transfer, photoelectrochemical

目 录

CONTENTS